Video Article

Isolation and Transplantation of Hematopoietic Stem Cells (HSCs)

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Abstract

Protocol

Total bone marrow preparation

1. Four B6 mice are sacrificed and dissected to obtain tibias, femurs, hip and spine.
2. During the dissections, the limbs and the bones are kept in PBS 2%FCS heat inactivated (optional 2mM EDA - dissection medium).
3. The clean bones are crushed with mortar and pestle in dissection medium. An efficient way to do it is to crush tibias, femurs and hips of each mouse, then 2 spines at a time.
4. The cell mixture obtained from each mouse is kept separate and filtered through a 40µm filter into a 50ml falcon tube. In this way, each tube contains the cell mixture obtained out of all the leg bones of a mouse, and half of the mixture obtained from 2 spines. At this stage, the filter is used to separate bone debris.
5. It is usually advisable to crush the leg bones twice to obtain white (with no bone marrow) bone fragments, and the spine fragments 2 or 3 times.
6. Fill up the tubes to have them balanced, and spin 5 min 1200rpm.
7. Aspirate the supernatant and loosen the pellet by dragging the tubes on the tube rack (this step is important after each centrifugation to minimize clump formation and cell loss). Resuspend each tube in 1 ml PBS 2% FCS (NO EDTA from now on) if you are doing lineage depletion, or in whatever volume is otherwise convenient for you.

Lineage depletion

1. This procedure allows you to eliminate highly differentiated cells and obtain a very heterogeneous population of cells, enriched for stem and progenitor cells. If you are sorting HSC, this step drastically reduces the number of cells that have to go through the cell sorting, therefore reducing sorting time and allowing you to sort HSC out of a higher number of mice, so that you can obtain a higher number of HSC in one single sorting.
2. If you are going to sort later, take out 50µl of cell mixture (I usually take a bit from each tube) for sorting controls (unstained and Sca1, c-Kit, CD48, Flk2 single color controls).
3. Add Lin cocktail 30ml/tube. Lineage cocktail contains a variety of antibodies, which unfortunately changes a little from lab to lab. In the Scadden lab, we use biotinilated antibodies against Gr1, Ter119, CD4, CD8, CD3, B220. Their final dilution in the staining is 1:700.
4. Vortex quickly to mix, and incubate for 15 min at 4°C.
5. During the incubation, prepare the columns on the magnets and 15ml elution tubes under the columns. Degas some PBS using a steriflip filter (you should see little air bubbles moving towards the surface of the PBS due to vacuum aspiration), and equilibrate the columns by applying 3ml degassed PBS to each column.
6. The column flow is about 1ml/5min, so it takes about 15 min to have the columns ready to use.
7. At the end of the incubation, add PBS 2% FCS to fill the tubes and spin down for 5 min at 1200rpm.
8. Aspirate the supernatant, loosen the pellets and resuspend in 1 ml/tube degassed PBS.
9. If you are sorting later, take out total bone marrow Lineage single color staining ctrl (15ml, a bit from each tube).
10. Add streptavidin beads 30ml/tube, vortex quickly to mix, and incubate for 15 min at 4°C.
11. At the end of the incubation, add 2 ml/tube of degassed PBS. Put new collection tubes under the columns, and apply each suspension to a column, filtering through a 40µm filter.
12. Add another 3 ml of degassed PBS to each tube to wash it. Once the columns are empty, apply again using the same 40µm filter.
13. The eluate contains the lineage depleted cell mixture, a heterogeneous population enriched for stem and progenitors cells. Pool the content of the 4 tubes into 2 tubes. Spin for 5 min at 1500rpm.
14. Aspirate supernatant. The pellet should be white or mainly white. Loosen the pellets and resuspend them together in 1ml total PBS 2% FCS if you are sorting later, otherwise resuspend in whatever volume is convenient for you.

LKS or long term HSC sorting

1. Take out lineage depleted single color staining ctrl (15ml). This will allow you to assess the efficiency of your lineage depletion.
2. The cell mixture to sort is stained in the 15ml tube.

Add antibodies:

Sca PE-Cy5.5	1: 100	10ml
Kit APC	1:100	10ml

SA PE-Cy7	1:500	2ml

To sort long term HSC, aslo add:

CD48 FITC	1:1000	1ml
Flk2 PE	1:200	5ml

Also, prepare single staining controls using the total bone marrow kept aside after the dissection and the total bone marrow lineage stained. These stainings can be done directly into FACS tubes.

Make sure to be in the dark!

1. Vortex quickly to mix and incubate at 4°C for 20 to 30 minutes, re-mixing half way through.
2. To wash away the excess of antibodies, fill up the tubes with PBS 2% FCS and spin for 5 min at 1500 rpm.
3. Remove supernatant, resuspend all pellets and move them to new FACS tubes with filter caps. It might take a while to get the cells to go through these filters, and it is important to apply some more PBS afterwards to wash the filter and minimize cell loss. This step is necessary to avoid clogging of the sorter, which is the worst nightmare of every cell sorter user.
4. When handing out your cells to the cell sorting facility, make sure to also prepare a tube with PBS 10% HI FCS where the cells will be collected.
5. If you are sorting LKS cells, you will obtain a heterogeneous population containing long term and short term repopulating HSC and multipotent progenitors. An average yield is 300,000 LKS from 4 mice.
6. If you have also used CD48 and Flk2 antibodies, you can separate each population of long term HSC, short term HSC and MPP. LT-HSC are the most rare population. An average yield is about 50,000 to 60,000 cells from 4 mice.

References